microbial pathogens) and parasitology (isolation and detection of protozoan parasites). No doubt many new processes and applications in other fields of biosciences and biotechnologies will be developed in the near future.

See Colour Plates 63, 64, 65, 66.

See also: **II/Centrifugation:** Analytical Centrifugation; Large-Scale Centrifugation. **III / Cells and Cell Organelles:** Field Flow Fractionation.

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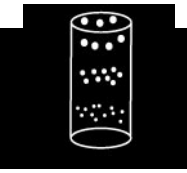
CELLS AND CELL ORGANELLES: FIELD FLOW FRACTIONATION



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Introduction

Analysis and sorting of living cells and purification of cell organelles are important procedures in the life sciences. There is a wide range of techniques and methodologies available, which can be divided into three main groups. The techniques in the first groups are based on physical criteria such as species size, density and shape, and include centrifugation, elutriation and field flow fractionation. Those in the second group are linked to cell surface characteristics, while flow cytometry techniques make up the third group. At a fundamental level, field flow fractionation (FFF) exploits the physical characteristics of the cells or cell organelles. However, cells or cell organelles exhibit some specific characteristics that can be described by a multipolydispersity matrix. The different physical characteristics of these biological materials require different FFF techniques and modes of operation. Special care must be taken if biological integrity and viability are to be preserved.

Specific Cell Characteristics

Cellular materials range in size from 1 μm to 50 μm. Cell populations are classified by a set of morphological, functional and biophysical characteristics. The biophysical characteristics are of particular interest in FFF. Usually, separations in FFF are influenced (but not directed) by surface properties of the sample components (to avoid particle–particle or particle–separator interactions). These properties can be modulated by the use of appropriate carrier-phase modifiers (surfactants). In terms of FFF separations, mass, size and density appear to be the major first order parameters. However, size is generally defined by the radius or the diameter of a sphere whose volume is identical to that of the cell. Size can therefore be deduced accurately if the cell of interest is perfectly spherical. However, this is not usually the case, and the sphericity index, I, is then used:

$$I = \frac{4.84(V^{2/3})}{S}$$

In this equation V is the cell volume and S is its surface area can be difficult to determine. In terms of cell population, these dimensions are averages and should be associated with a variance. These general

considerations are also valid for cell density. Each measurable cell characteristic is therefore associated with an average value and its related variance. A probe of this 'diversity' is of great significance, even in a highly purified cell population. Polydispersity is historically defined in polymer chemistry as the ratio of the percentage of the standard deviation to the average value.

Any cell population can therefore be described by a set of values (average, variance) that leads to a polydispersity index. Cells are different from polymers in that each measurable characteristic of a cell can be associated with polydispersity, so polydispersity can apply to mass, size, volume or density. This matrix of parameters (average, variance) can be used to define a 'multipolydispersity matrix'. The dimensions of the multipolydispersity index depend on the information available. For example, the human normal red blood cell (HNRBC) is the best known cellular material. Its average volume has been measured as 95 ± 5 fL, and its surface area has been calculated as $138.0 \pm 5\ \mu m^2$. The HNRBC is known for its discoid shape, which generates other measurable dimensions. Its average diameter is $8.1 \pm 0.43\ \mu m$, and minimum and maximum thicknesses of $1.0 \pm 0.3\ \mu m$ and $2.4 \pm 0.15\ \mu m$ have been measured. An average sphericity index of 0.77 has been calculated. The density was found to vary from 1.035 to 1.102, with an average value of 1.051. Using these data, it is possible to describe the HNRBC population using a preliminary multipolydispersity matrix, as shown in **Table 1**.

More complex properties such as cell/nucleus ratio or surface electric charge density (ζ potential, used for dielectric cell purification) can later be added to this basic matrix. In FFF, the sample (i.e. cell surface) characteristics are most important in the development of any separation process. The separator material is chosen so as to avoid or limit particle–separator interactions, which can lead to limited recovery and viability, separator ageing or poisoning. In this domain a general set of empirical rules emerged. If the cell surface is hydrophilic (blood and yeast cells) a hydrophobic material should be used for the separator. The lower the surface energy of the cell, the less non-specific cell–separator interactions occurred, and vice versa. The interactions between the environmental material and the cells can now be assessed by the general 'biocompatibility' rules.

Table 1 Multipolydispersity matrix for HNRBC

	Average	*Standard deviation*	*Polydispersity (%)*
Volume (fL)	95	5	10
Density	1.051	0.08	3
Sphericity	0.77	0.15	11

However the HNRBC population found in circulating blood is not a homogenous, and contains red blood cells (RBCs) of different ages. This suggests that a morphologically homogenous population can be considered to contain different sub-populations. An HNRBC suspension contains cells of equal volume, and cells of equal density, shape or mass. This complex definition of 'population' characteristics is described in **Figure 1**A. If a set of common parameters is defined, the HNRBCs that meet these limitations may represent only a minor proportion of the whole population. By considering some aspects of this multipolydispersity matrix, rules for cell separation based on biophysical characteristics can be found. **Figure 1**B shows a three-dimensional graphical representation of the multipolydispersity of the circulating blood cells (platelets, HNRBC, lymphocytes, monocytes, granulocytes) based on the characteristics of size and density. It is obvious that if a size driven cell separation technology could be used, all these populations could be isolated. An isolation technique based only on density would be more complex, as some blood cell population densities overlap. However a size and density driven separation (whose balance has not so far been assessed) would allow selective isolation.

FFF Techniques for Cell Separations

The Different Techniques

The requirements of different fields have led to a wide variety of FFF techniques. Four major technologies have emerged. The first uses fields generated by gravity, either simple earth gravity or centrifugally generated multigravitational fields. It is now generically described as sedimentation FFF (SdFFF for multigravity fields and GFFF for gravity induced fields). The basic technology is the same, although a more complex instrumental design is required for SdFFF, as shown in **Figure 2**A and B. However, the very simple GFFF designs and procedures can be upgraded for SdFFF separations. This group was historically the most successful in biological applications. Channel cleaning, decontamination and sterilization procedures are common and can be performed simply, as described later. There are many channel wall materials available, and a wide diversity of sample injection technologies or procedures.

The second group encompasses fields generated by a flow, where the accumulation wall is made of a semipermeable membrane of adapted cut-off. This

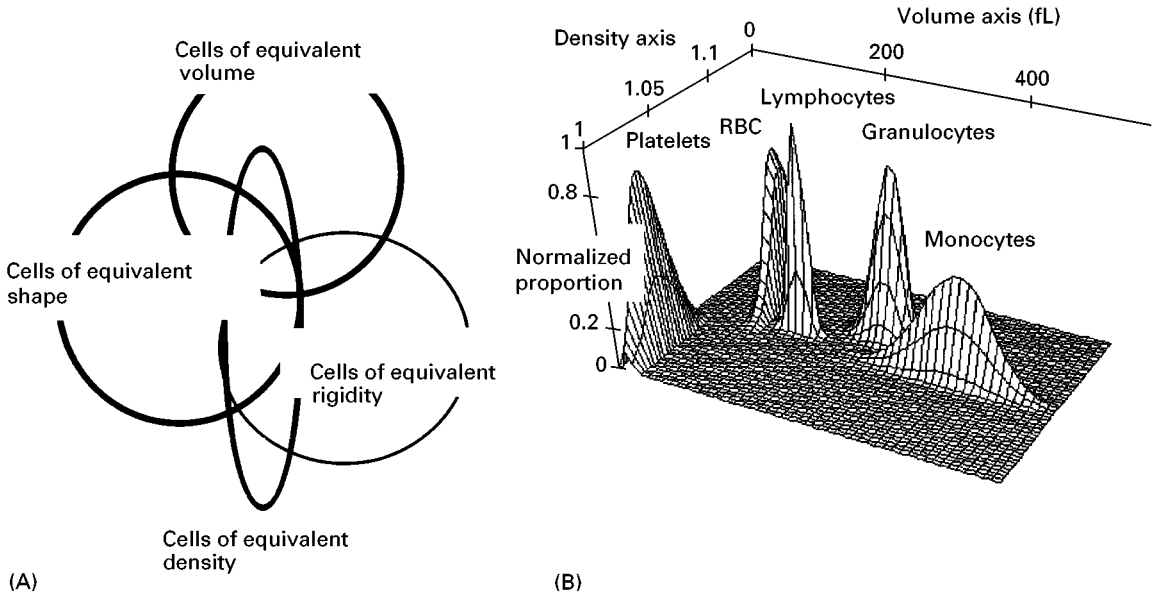

Figure 1 Multipolydispersity matrix. (A) The definition of a homogenous population is complex. A cell population with homogenous morpological characteristics is actually a complex mixture of sub-populations with different biophysical characteristics. (B) Three-dimensional plot of circulating blood cells (normalized dimensions) as a function of size and density (from bibliographic data). Red blood cells and lymphocytes have the closest characteristics.

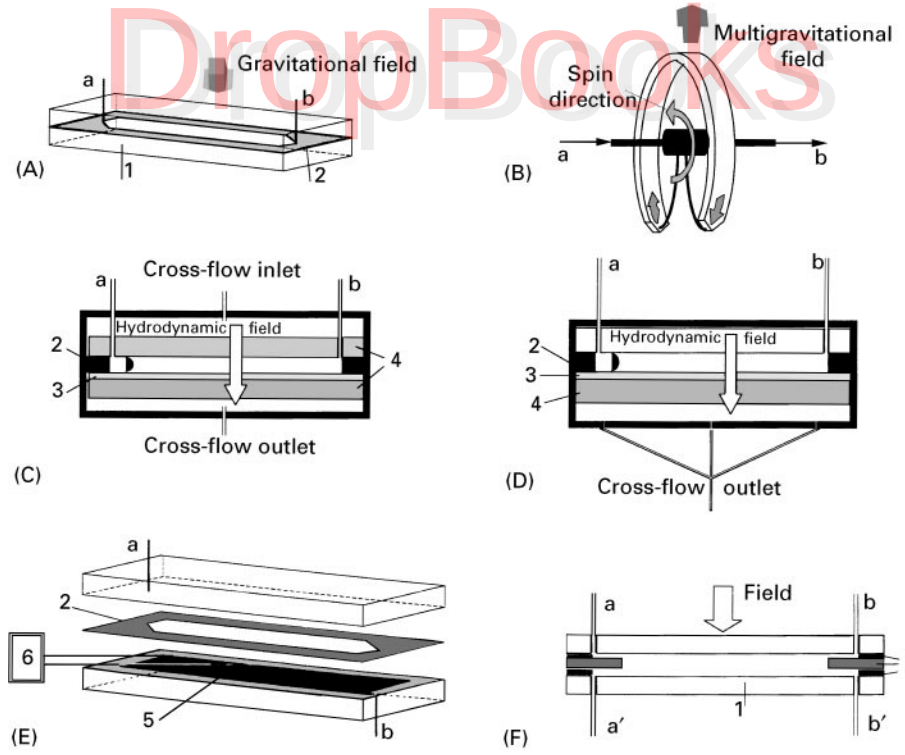

Figure 2 The different FFF techniques. (A) Gravitational FFF device; (B) Sedimentation FFF device; (C) symmetrical flow FFF device; (D) asymmetrical flow FFF device; (E) experimental layout of DEP-GFFF device; (F) schematic view of SPLITT-FFF device. 1, Channel wall (accumulation wall); 2, Mylar® spacers; 3, semipermeable membrane (accumulation wall); 4, frit; 5, interdigitated electrode; 6, power amplifier and source of signal; a, inlet (sample and carrier phase); b, outlet (to detector and fraction collection); a' and b', secondary inlet and outlet.

group is of interest because of the possibility of separating species by buoyancy (particle suspension and carrier phase with identical density), such as cell organelles or macromolecules. Two flow-generated FFF techniques were developed. Symmetrical flow FFF (FFFF) uses two independent flows, one to create the external field and the second to sweep the species along the channel (**Figure 2**C). Asymmetrical flow FFF (AFFFF) uses the same flow along and across the channel, as shown in **Figure 2**D. However, sample injection procedures are more limited and complex membrane–sample interactions may occur. Fortunately, the wide range of membranes available has made the technique very versatile. The in-channel pressure needed to create the transverse flow-generated field by means of off-channel restrictors has led to rather sophisticated fraction collection devices.

The third group uses electric fields either as the main transverse field (electric FFF) or as dielectric fields combined with gravity (DEP-GFFF) to obtain a hyperlayer focusing elution mode, as shown in **Figure 2**E. The fourth group encompasses continuous separation devices based on the general SPLITT concept, as shown in **Figure 2**F.

General Features Linked to Cell Elution Process

The osmotic and pH properties of the biological media must be preserved. Buffer solutions of known pH and ionic strength are used. For example the classical isotonic phosphate buffered saline (PBS) solution. Compared with other FFF elution carried phases, only limited surfactants can be used. Sucrose solution and albumin are both possibilities, the latter being the most versatile carrier phase modifier, usually at a concentration of 0.1% (v/w). The surfactant effect of albumin limits particle–particle interactions as well as particle–wall ones. Albumin adsorption occurs, on relatively hydrophobic channel wall materials, leading to channel ageing or poisoning. Rigorous cleaning and channel regeneration procedures are therefore recommended.

As the general purpose of cell elution in FFF is to limit particle–wall interactions, the commonly used 'stop flow injection procedure' can cause problems. The procedure involves injecting sample at the inlet of the channel in the absence of a flowing stream but while an external field is applied. The species are concentrated near the accumulation wall at the beginning of the channel. The procedure is essential if species are eluted according to the 'Brownian' elution mode, but this is not the case for cells. With cells the risk of generating particle–particle or particle–wall interactions is increased. Specific instrumental modifications of the inlet geometry (directly on the accumulation wall) or low flow-injection procedures will limit the risk. However, flow injections may enhance selectivity because of the different speeds at which sample components reach the steric hyperlayer focused position in the channel thickness. Elution of cellular material in a steric mode is associated with reduced recovery and possibly with reduced selectivity. Examples of the steric hyperlayer elution mode of HNRBCs and duracytes are given in **Figure 3**.

Cell Elution Methodology and Detection

Decontamination and Sterilization of FFF Separator

This is necessary to prevent bacterial contamination, but the use of sodium azide should be avoided as it is a cellular toxic. Safe and effective decontamination processes can be considered as an additional step in the cleaning procedure.

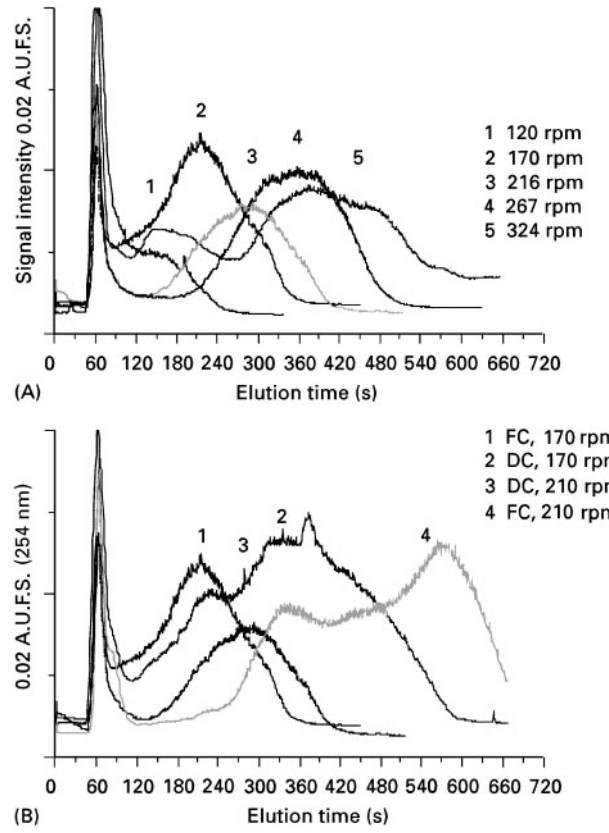

Figure 3 Red blood cells with different elution characteristics in sedimentation field flow fractionation. (A) Fractograms (elution signal) of fresh RBCs. Channel walls made of hydrophobic materials, channel dimensions 0.5 cm wide, 0.025 cm thick, 58 cm long. The channel distance from rotor axis, 10.7 cm. Photometric detection at 254 nm. Sample injection in the established flow (flow rate of 0.5 mL min^{-1}). (B) Fractograms showing retention differences between fresh RBCs and duracytes. RBCs and duracytes are similar in size, but duracytes are rigid fixed RBCs of higher density. FC, fresh red blood cell; DC, duracytes.

The cleaning procedure involves flushing the channel (ten times the void volume) with a hypo-osmotic carrier phase to destroy the cells, then with a classical deproteinization agent (ten times the void volume) to desorb and destroy the cell and surfactant proteins adsorbed in the FFF separator. Finally the system is flushed again with the hypo-osmostic carrier phase.

Decontamination is performed by allowing a hypochloride solution (3–6°C) to flow through the whole FFF system. The volume used should be three to four times the void volume of the FFF device. Prior to injection the channel should be flushed with ethanol and rinsed with the sterile mobile phase. Decontamination and sterilization can be simultaneous if a 5°C hypochloride solution is used with a 70°C ethanol solution.

Cell Detection, Viability and Recovery

At the outlet of the channel it is necessary to obtain eluted material that retains its integrity, that is the diagnosis of the whole particle. Photometric devices operated in the light scattering mode can be used to follow elution. After fraction collection off-line analyses methods such as microscopy, granulometric or flow cytometry analyses are recommended. Cell specific staining is also possible. Cell viability can be diagnosed by means of specific tests, and recovery must also be clearly defined.

Conclusions

The choice of FFF separation techniques for purification of cell or cell organelle populations is influenced by their possible elution modes. Micron sized species eluted under the steric hyperlayer model can be separated using sedimentation techniques if their density differs from that of the carrier phase medium. This is also possible for 'nonbuoyant' sub-micron sized cell organelle species eluted according to the 'Brownian' elution mode.

If species are to be separated according to their size, FFF techniques that use a flow generated external field are preferred. If differences in surface characteristics are important, electrical-based FFF techniques should be chosen. For large sample volume preparations, SPLITT techniques are useful, whatever the external field. The major technical goal in cell separation is not the set-up of the separator, but the construction of a chain of knowledge. The separator is designed according to the sample characteristics as well as the mode of operation. Cleaning and sterilization procedures, and detection and viability procedures, are linked to the nature of the material to be purified. This 'biotechnological' process is of major importance for FFF separations in the life sciences.

See also: **II/Particle Size Separation:** Field Flow Fractionation: Electric Fields; Theory and Instrumentation of Field Flow Fractionation. **III/Colloids:** Field Flow Fractionation. **Polymers:** Field Flow Fractionation. **Proteins:** Field Flow Fractionation. **III/Catalyst studies: Chromatography: Isolation: Magnetic Techniques**.

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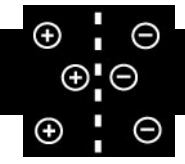
CHELATING ION EXCHANGE RESINS



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Introduction

The useful role of ion exchange is to separate a solution at a low initial concentration C_i into a small